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EFFECT OF HOST FASTING AND SUBSEQUENT REFEEDING ON THE GLYCOGEN METABOLIZING ENZYMES IN *HYMENOLEPIS DIMINUTA* (CESTODA)

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ABSTRACT

During host fasting, total glycogen synthase activity in *Hymenolepis diminuta* increased to a maximum at 48 h and decreased thereafter. The activity ratio of glycogen synthase I to total glycogen synthase (I/T), however, decreased before rising again. The increase in the active form of glycogen synthase after 48 h of host fasting might prevent complete exhaustion of glycogen during prolonged starvation. The total and active glycogen phosphorylase activities increased throughout the whole fasting period.

Twenty-four hours after refeeding the fasted host, the glycogen synthase and phosphorylase activities in *H. diminuta* decreased. *In vitro* studies, however, revealed an immediate increase in the glycogen synthase activity when glucose was available.

Protein content of worms artificially reduced in length, from normal hosts was comparable to that of the 48 h starved worms but not to their glycogen content, glycogen synthase, and phosphorylase activities.

INTRODUCTION

Glycogen, the major carbohydrate reserve, serves as an important energy source for *Hymenolepis diminuta* (Read, 1972; Mied and Beuding, 1979). Glycogen synthase (UDP-glucose:glycogen-4-glycosyl transferase EC 2.4.1.11) and glycogen phosphorylase (α-1,4-glucan-orthophosphate glucosyl transferase EC 2.4.1.1) are, therefore, the two main regulatory enzymes critical to the energy metabolism of the worm.

Effect of host fasting on the glycogen synthase activities in *H. diminuta* has been determined. Fasting the rat host for 24 h decreases the active form of the enzyme. Its activity, however, greatly increases one hour after the host is refed. (Mied, 1975; Dendinger and Roberts, 1977). However, the correlation between the activities of glycogen synthase and glycogen phosphorylase in *H. diminuta* during fasting and refeeding have not been determined. Thus the present investigation was undertaken to elucidate the regulatory mechanism of these two enzymes in *H. diminuta* in response to physiological changes of fasting and subsequent refeeding of the host.

MATERIALS AND METHODS

Male Sprague-Dawley rats weighing 75–125 g were force-fed 12 cysticercoids of *Hymenolepis diminuta* reared in adult *Tenebrio* spp. Before and after infection, the rats were fed Purina Laboratory Chow and water *ad lib.* Twenty-four-day-old worms in hosts fasted for various periods were flushed from excised gut with saline solution (Ip and Fisher, 1982a) containing 120 m*M* NaCl, 5 m*M* KCl, 1 m*M* $CaCl_2$, 1 m*M*

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$MgCl_2$, 10 m*M* $NaHCO_3$, 7.5 m*M* Na_2HPO_4, and 2.5 m*M* KH_2PO_4 equilibrated to pH 7.4 with 5% carbon dioxide in nitrogen. The worms were removed from their host between 0700 and 0900 h during each experiment. The wet weight of the worms was obtained using a Sartorius Electronic Semimicro Balance 2024 MP 6. For the determination of wet weight: dry weight ratio (WW/DW), the weighed worms were dried in the oven at a temperature of 95°C for 24 h, after which the dry weight was recorded.

For the determination of glycogen and protein content and enzyme activities, the weighed worms were homogenized in an ice-cold buffer containing 50 m*M* Hepes (N-2-hydroxy-ethyl-piperazine-N-2-ethane sulfonic acid) buffer (pH 7.4), 5 m*M* ethylenediamine tetraacetic acid (EDTA), 50 m*M* NaF, and 40 m*M* 2-mercaptoethanol (βME). The samples were then centrifuged at 25,000 × *g* for 10 min at 4°C. The supernatant fluid was maintained at ice-cold conditions before various assays were performed.

Enzyme assays

Glycogen synthase. The activity of glycogen synthase was determined by measuring the incorporation of UDP-(^{14}C)glucose (Amersham) into glycogen according to Villa-Moruzzi *et al.* (1979). For total activity determination, 20 μl of the supernatant fluid was introduced into an assay mixture containing 50 m*M* Hepes (pH 7.4), 1% *H. diminuta* glycogen, 6.7 m*M* UDP-(^{14}C)glucose (0.25 μCi/μM), and 10 m*M* glucose-6-phosphate in a total volume of 80 μl. For glycogen synthase I determination, glucose-6-phosphate was omitted from the assay mixture. Radioactivity was determined by using Biofluor (NEN) and a Packard Tri-Carb 300 liquid scintillation spectrometer. One unit of enzyme activity is defined as the incorporation of one μmole of (^{14}C)glucose from UDP-(^{14}C)glucose into glycogen per minute at 30°C (Donahue *et al.*, 1981).

Glycogen phosphorylase. The activity of glycogen phosphorylase was determined in the direction of glycogen synthesis (Gilboe *et al.*, 1972). For total activity determination, 20 μl of the supernatant fluid was introduced into an assay mixture containing 10 m*M* (^{14}C)glucose-1-phosphate (Amersham) (0.30 μCi/μM), 1% *H. diminuta* glycogen, 25 m*M* imidazole, 1 m*M* EDTA, 40 m*M* βME (pH 6.1), and 1 m*M* 5′-AMP in a total volume of 80 μl. For phosphorylase 'a' determination, 5′-AMP was omitted from the assay mixture. One unit of enzyme activity is defined as the incorporation of one μmole of (^{14}C)glucose from (^{14}C)glucose-1-phosphate into glycogen per min at 30°C (Donahue *et al.*, 1981).

Glycogen purification

Glycogen required for the above assays was purified with methanol according to Donahue *et al.* (1981). The purity of the lyophilized glycogen was determined according to Montgomery (1957). Preparations of at least 95% purity with no indication of the presence of protein and inorganic phosphate were used.

Glycogen and protein assay

To determine the glycogen level of the tissue extracts, samples were immediately transferred into boiling water after centrifugation to stop all enzymatic reactions. The glycogen was then precipitated and purified using the method of Good *et al.* (1933) and assayed using the method described by Montgomery (1957) and Roehrig and Allred (1974) with bovine liver glycogen (Sigma Co.) as the standard.

TABLE I

Water content of H. diminuta *under different experimental conditions*

Host's condition	n	WW/DW ratio	Water content (%)
No fasting	3	4.45 ± 0.19	77.9 ± 0.8
24 h fasting	3	4.18 ± 0.12	76.4 ± 0.3
48 h fasting	2	4.61 ± 0.34	78.3 ± 1.6
72 h fasting	3	4.29 ± 0.76	72.9 ± 3.6
96 h fasting	2	4.25 ± 0.87	72.2 ± 4.1
No fasting*	4	4.51 ± 0.19	77.8 ± 0.9

* Worms from unfasted host reduced artificially to size of those obtained from 48 h fasted host.

The protein content in the extract was determined according to Bradford (1976). Standard used was bovine serum albumin (Sigma Co.).

RESULTS

There was no significant change ($P > 0.05$) in the wet weight:dry weight ratio of *H. diminuta* from unfasted as well as fasted hosts (Table I). Since the worm's water content remained relatively constant regardless of the experimental conditions applied, the protein and glycogen content as well as the enzyme activities were expressed in terms of the wet weight (WW) of the worm.

The protein contents increased significantly ($P < 0.05$) during a 96-h fast (Table II). Upon refeeding, the protein content decreased when compared to that of worms from fasted hosts (Table IV). As the protein content fluctuated with different experimental conditions, it was not suitable for use as a traditional reference to express enzyme activity.

We attempted to assay the worms' glycogen content using the method described by Roehrig and Allred (1974) which specifically measures glycogen enzymatically and avoids potential errors in extraction and subsequent precipitation. However, the above method was unsuitable as it was affected by βME present in the homogenizing buffer which is necessary to minimize phosphoglucomutase activity. Therefore, the

TABLE II

Effect of host fasting on glycogen contents, protein contents, and glycogen synthase activities of H. diminuta

Host's condition	n	No. of worms	Protein (% ww)	Glycogen (% ww)	Glycogen synthase activity*			
					Total (T)	I	D	Ratio I/T
No fasting	5	12 ± 1	1.8 ± 0.5	3.0 ± 1.0	966 ± 45	71 ± 45	895 ± 62	0.073
18 h fasting	2	10 ± 4	2.0 ± 0.5	1.8 ± 0.5	903 ± 98	37 ± 1	866 ± 98	0.041
24 h fasting	3	10 ± 1	2.3 ± 0.9	1.6 ± 0.4	1327 ± 318	72 ± 24	1254 ± 273	0.054
48 h fasting	3	7 ± 2	3.7 ± 0.4	0.6 ± 0.4	2175 ± 529	250 ± 97	1926 ± 422	0.115
72 h fasting	2	3 ± 0	4.4 ± 0.3	0.36 ± 0.01	1752 ± 493	161 ± 77	1592 ± 416	0.092
96 h fasting	2	3 ± 2	6.9 ± 0.3	0.49 ± 0.04	1413 ± 540	333 ± 70	1081 ± 610	0.236
No fasting**	1	12	3.3	4.1	1203	33	1170	0.027

* Unit: activity per gm (ww) worm.

** Worms from unfasted host reduced artificially to size of those obtained from 48 h fasted host.

TABLE III

Effect of host fasting on glycogen phosphorylase activities of H. diminuta

Host's condition	n	Glycogen phosphorylase activity*			
		Total (T)	a	b	Ratio a/T
No fasting	5	229 ± 60	183 ± 57	46 ± 15	0.799
18 h fasting	2	175 ± 45	151 ± 44	24 ± 1	0.863
24 h fasting	3	270 ± 166	210 ± 153	59 ± 21	0.778
48 h fasting	3	311 ± 83	222 ± 59	90 ± 24	0.714
72 h fasting	2	414 ± 33	397 ± 72	59 ± 74	0.959
96 h fasting	2	630 ± 72	469 ± 21	161 ± 96	0.744
No fasting**	1	635	416	219	0.655

* Unit: activity per gm (ww) worm.

** Worms from unstarved host reduced artificially to size of those obtained from 48 h fasted host.

traditional method of glycogen extraction by Good *et al.* (1933) and Montgomery's (1957) method of glycogen analysis were chosen since the majority of the polysaccharide present in *H. diminuta* were glycogen (Read, 1972; Dendinger and Roberts, 1977, Mied and Beuding, 1979). After extraction, no significant difference was observed between the results obtained by using the methods of Montgomery (1957) and those of Roehrig and Allred (1974). The percentage recovery of the extraction procedure was estimated to be 98.12 ± 0.23 (n = 4) when such method was applied to 100 mg of standard bovine liver glycogen (Sigma Co.). The glycogen levels in *H. diminuta* decreased significantly ($P < 0.05$) during a 48-h fast and leveled off thereafter (Table II). When the host was refed, the worm's glycogen level increased significantly ($P < 0.05$) (Table IV).

Glycogen synthase and phosphorylase activity in H. diminuta *during host fasting and refeeding*

The total glycogen synthase activity of *H. diminuta* increased significantly ($P < 0.05$) during the first 48 h of fasting with a subsequent significant decrease ($P < 0.05$) at 96 h (Table II). The ratio of glycogen synthase I activity to total activity (I/T), however, decreased before rising again. When the host was refed after starvation, there were decreases in both the worm's total glycogen synthase activity and the I/T ratio (Table IV).

During fasting, the total as well as the active 'a' form of glycogen phosphorylase increased significantly ($P < 0.05$) (Table III). After refeeding, the phosphorylase activity decreased (Table V).

Effect of artificially reducing the size of H. diminuta *on its enzyme activities*

When the host was fasted, there was not only a decrease in the number of worms in the host (Table II) but also a reduction of their length. Therefore an experiment was performed to determine if the changes in enzyme activities reported above were biochemical adaptations to the starving conditions or were merely due to the uneven distribution of enzymes along the length of the parasites.

Unstarved worms were artificially shortened by cutting with a pair of scissors to the approximate length of those obtained from the 48 h starved host. Their protein

TABLE IV

Effect of in vitro *refeeding of the host after fasting on the protein contents, glycogen contents, and the glycogen synthase activities in* H. diminuta

Host's condition	No. of worms	Protein (% ww)	Glycogen (% ww)	Glycogen synthase activity*			
				Total (T)	I	D	Ratio I/T
No fasting	12	1.6	2.9	942	70	872	0.074
24 h fasting + 24 h refeeding	11	1.5	2.8	863	27	835	0.031
48 h fasting + 24 h refeeding	12	3.1	2.5	1742	65	1676	0.037
96 h fasting + 24 h refeeding	6	2.4	4.8	1198	40	1158	0.034

* Unit: Activity per gm (ww) worm.

and glycogen contents as well as glycogen synthase and phosphorylase activities were examined. The amount of protein (3.3%) present in these worms was comparable to that of the 48 h fasted ones but the glycogen content (4.1%) was significantly higher ($P < 0.05\%$) (Table II). The glycogen synthase activity in these worms was lower than these obtained from the fasted hosts while the phosphorylase activity was higher (Table II and III).

In vitro *refeeding studies on the enzyme activities*

The immediate effect of refeeding on the enzyme activities cannot be determined under *in vivo* conditions as food fed to the host requires several hours to become available to the parasites. An *in vitro* study thus was performed. Twenty-four-day-old worms from five rats fasted for 48 h were randomized and sorted into groups of seven,

TABLE V

Effect of in vitro *refeeding of host on the glycogen phosphorylase activities of* H. diminuta

Host's condition	No. of worms	Glycogen phosphorylase activity*			
		Total (T)	a	b	Ratio a/T
No fasting	9	237	135	102	0.570
24 h fasting + 24 h refeeding	11	173	141	32	0.815
48 h fasting + 24 h refeeding	12	266	229	37	0.890
96 h fasting + 24 h refeeding	6	210	130	80	0.619

* Unit: activity per gm (ww) worm.

TABLE VI

In vitro *refeeding studies of the protein contents, glycogen contents, and the activities of glycogen synthase in* H. diminuta *from 48 h starved host*

Host's condition	No. of worms	Protein (% ww)	Glycogen (% ww)	Glycogen synthase activity*			
				Total (T)	I	D	Ratio I/T
No fasting	12	1.6	2.90	942	70	872	0.074
48 h fasting	7	3.9	0.80	2135	245	1890	0.115
48 h fasting + 10 min refeeding	7	4.2	0.36	4288	3034	1254	0.708
48 h fasting + 20 min refeeding	7	3.4	0.20	3916	2869	1046	0.733
48 h fasting + 1 h refeeding	7	2.6	0.18	2375	1771	604	0.746
48 h fasting + 3 h refeeding	7	2.7	1.80	2021	520	1501	0.257

* Unit: Activity per gm (ww) worm.

each group constituting a sample. Each sample was incubated in 20 ml of saline solution containing 1 m*M* glucose equilibrated with 5% CO_2 in nitrogen. The samples were incubated at 37°C in a water-bath shaking at 60 oscillations/min for various periods. Saline was changed once every hour to prevent accumulation of metabolic end products and to replenish glucose.

The results (Table VI & VII) indicated an increase in the glycogen content of the worms incubated in a medium of 1 m*M* glucose. Immediately upon the availability

TABLE VII

In vitro *refeeding studies of the activities of glycogen phosphorylase in* H. diminuta *from 48 h starved host*

Host's condition	No. of worms	Glycogen phosphorylase activity*			
		Total (T)	a	b	Ratio a/T
No fasting	9	237	135	102	0.570
48 h fasting	7	334	231	103	0.692
48 h fasting + 10 min refeeding	7	76	44	32	0.579
48 h fasting + 20 min refeeding	7	81	48	34	0.593
48 h fasting + 1 h refeeding	7	77	39	38	0.506
48 h fasting + 3 h refeeding	7	105	54	51	0.514

* Unit: activity per gm (ww) worm.

of glucose, the total glycogen synthase activity increased while the total phosphorylase activity decreased. There was also an abrupt increase in the I/T ratio of glycogen synthase activities.

Discussion

Fasting the host not only significantly decreases the wet weight and length of *H. diminuta* (Read and Rothman, 1957), it also increases the protein concentration (Goodchild, 1961a) and decreases the glycogen content in the worm (Read and Rothman, 1957; Goodchild, 1961b). The present investigation confirms the above reports (Table I). The apparent increase in protein content in the worm during host fasting may be due to the uneven distribution of non-protein reserve substances in the worm as suggested by Goodchild (1961b). The older reproductive proglottids, which are always the first to be shed during starvation, contain relatively more lipids and polysaccharides (Ip and Fisher, 1982b). This is confirmed by the present observation that the protein contents of the 48-h fasted worms and the unfasted worms artificially reduced to equivalent length are comparable.

When nutrients become limiting during host fasting, the parasite has to metabolize its endogenous glycogen to survive. The glycogen content, therefore, decreases with increasing periods of fasting (Read, 1956; Goodchild, 1961a). However, it leveled off after fasting for 48 h, suggesting that a minimal concentration of glycogen was maintained even under extreme conditions. Since approximately 10% of the worm's glycogen is structural (Beuding and Fisher, 1970), it would be disadvantageous for the worm to utilize all of them. Furthermore, a minimal amount of glycogen is required to serve as primer should food again become available. The marked increase in glycogen content 24 h after refeeding of hosts subjected to various periods of fasting (Table II, IV) suggests that there is a proportionate increase in the efficiency of glycogenesis with starvation. This is in agreement with observations made by earlier investigators (Daughtery, 1956; Read, 1956; Read and Rothman, 1957). The same rapid conversion of glucose to glycogen also is observed in the fasted surrenalectomised rats after feeding with glucose (Cori and Cori, 1927).

In the present study, the total glycogen synthase activities in *H. diminuta* increased during host fasting but the active 'I' form decreased simultaneously. The large amount of inactive glycogen synthase present would act as a reserve which can be rapidly converted to the active form once food is available. Such conversion would be more efficient than *de novo* synthesis of the enzyme. Similar phenomena have been observed in the rat liver (Gruhner and Segal, 1970; Curnow and Nuttall, 1972). The trend, however, reversed itself in worms which had been fasted for more than 48 h; the total synthase activities decreased while the activities of the 'I' form increased (Table II). The total phosphorylase activity as well as the 'a' form, unlike the rat-liver system, increased during host fasting (Table III). Such increase in the activity of 'I' form of synthase and 'a' form of phosphorylase suggests that the synthesis and degradation of glycogen occur simultaneously. As the amount of glycogen present in worms fasted for more than 48 h was very small, the increased activity of the synthase helped to recycle the glucose released by phosphorylase back into glycogen. Such a mechanism prevents the glycogen from being completely used up during prolonged fasting. This explains the leveling off of the glycogen content after 48 h of host starvation. Similar increase in the 'I' form of glycogen synthase has also been reported in the rat (Curnow and Nuttall, 1972; Hue *et al.*, 1975).

Twenty-four hours after refeeding rats which have been fasted for various periods, the activity of glycogen synthase I in *H. diminuta* decreased as compared to those of

starved worms. This agrees with observations made on the rat liver (Hornbrook, 1970; Curnow and Nuttall, 1972) but contradicts the results reported by Mied (1975) for the same species of tapeworm. This apparent contradiction may be due to the fact that synthase activities in the present study was measured 24 h after food was made available to the starved rats. Mied (1975) measured the activity immediately after refeeding. *In vitro* refeeding studies however, demonstrated that there was indeed a marked increase in the total synthase activity immediately upon incubation in 1 m*M* glucose indicating that glucose perhaps could be the activator of glycogen synthase in *H. diminuta*. Glucose-induced increase in synthase activity also has been reported for isolated rat livers perfused with glucose, and it appears to be independent of changes in the levels of exogenous hormones (Buschiazzo *et al.*, 1977). Upon further incubation in the presence of glucose, the glycogen synthase activity gradually dropped (Table III). As glycogen accumulates in the worm, the average molecular weight of glycogen increases (Orrell *et al.*, 1966) and the presence of high molecular weight glycogen molecules in the worm inhibits glycogen synthase activity, especially that of the 'I' form (Mied, 1975; Dendinger and Roberts, 1977).

A decrease in both the total and the 'a' form of the phosphorylase activities in *H. diminuta* was observed 24 h after the host was refed. There was also a steep drop in the phosphorylase activity in worms incubated in 1 m*M* glucose after 48 h of starvation. Such decrease in phosphorylase activity suggests that glucose may deactivate the phosphorylase. In mammalian systems this deactivation is effected by the glucose binding to the 'a' form of the enzyme, forming a complex which is a superior substrate for phosphorylase phosphatase. This lowers the phosphorylase activity which in turn slows down glycogenolysis. The removal of phosphorylase 'a', which is a strong inhibitor of glycogen synthase phosphatase, allows the latter enzyme to convert the synthase 'D' into its 'I' form, which determines the ultimate rate of glycogen synthesis (Stalmans *et al.*, 1974; Hers, 1981). Simultaneous deactivation of phosphorylase 'a' and activation of the 'I' form of synthase in *H. diminuta* were observed in the *in vitro* refeeding studies (Tables VI, VII). Whether the deactivation of phosphorylase 'a' is a prerequisite to the activation of glycogen synthase, as proposed by Stalmans *et al.* (1974) for the mammalian system as yet cannot be confirmed.

Glycogen content, glycogen synthase, and phosphorylase activities of artificially reduced worms from unfasted hosts were different from that of normal worms from unfasted hosts indicating differential distribution of the above substances along the length of the worms. When worms from 48 h fasted hosts were compared with worms from unfasted hosts artificially shortened to the length of the former, the glycogen content as well as the enzyme activities were also found to be different (Tables II, III). Such differences cannot be due to the shedding of proglottids alone but is attributable to a biochemical adaptation to starvation.

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